

Pipette Procedures and Calibration

APPLICATION

This procedure describes how to calibrate and use several different types of laboratory pipettes. Pipettes are designed for the transfer of known volumes of liquid from one container to another. There are three types of pipettes: volumetric, measuring, and the mechanical micropipette.

PIPETTE USE

The user shall ensure the pipette is calibrated and that the calibration brackets the intended volume to pipette. The following instructions apply specifically to the use of transfer (volumetric) pipettes, but with minor modification may also be used for other types of pipettes as well.

Liquids are drawn into the pipette by application of a slight vacuum. Do not use your mouth as a suction source. The laboratory has a variety of suction devices that can be used in pipette procedures. Make sure that these devices are clean and dry. If liquid is accidentally drawn into the suction device, it should be immediately disassembled, cleaned, and dried.

Keep the tip of the pipette below the surface of the liquid when drawing liquid into the pipette. Fill the pipette above the graduation line. Make certain there are no air bubbles in the liquid or foam at the surface. Discontinue the suction when the liquid is above the graduation mark. Tilt the pipette slightly from vertical and remove any liquid on the outside of the pipette tip with clean tissue. While holding the pipette in a vertical position, release pressure to allow the meniscus to slowly approach the graduation mark. When the meniscus reaches the graduation mark, remove the drop of liquid on the tip of the pipette by touching it to the wall of the container. Recheck the position of the meniscus. If it is not at the center of the calibration line, carry out the necessary steps to bring it to that point, by raising the level of the liquid in the pipette. After pipetting the liquid into the receiving container, touch the pipette tip to the side to remove any droplet adhering to the tip.

CALIBRATION OF AUTO PIPETTES

Pipettes require frequent calibration to ensure that their mechanical parts have not suffered damage that may cause changes in the volume of liquid delivered by the pipette. This calibration should be accomplished monthly or anytime the performance of the pipette may be questionable. Calibration of pipettes is accomplished by repeatedly weighing aliquots of distilled water delivered by the pipette. The average weight is calculated as well as the standard deviation. From these two values, the coefficient of variation is calculated and used to estimate the accuracy and precision of the micropipette. The procedure for this determination is provided below. This procedure should bracket the volumes the pipette can deliver.

Tare a small plastic weighing boat on the analytical balance. Depress the plunger on the pipette until it just stops from resistance, place the tip of the pipette in the beaker of water, and slowly release the plunger to its fully

Pipette Procedures and Calibration

extended position. This will draw an aliquot of water into the pipette tip. Note: The exact procedures may vary from one manufacturer to another. Remove excess water on outside of pipette tip and deliver the aliquot of water to the weighing boat by fully depressing the plunger on the pipette until it stops.

Record the weight of the pipette-delivered aliquot on a Pipette Calibration Form. Repeat procedure of weighing aliquots of water three times using the same pipette tip.

Calculate the average and standard deviation of all weighed aliquots of water.

Calculate the coefficient of variation (CV) of the pipette using the following formula:

$$CV = \text{Standard Deviation in Volume} / \text{Average Volume} \times 100$$

If the CV is less than or equal to $\pm 3\%$, the pipette is acceptable for use. If the CV is greater than 3%, the pipette shall not be used and must be set aside for repair and cleaning, or discarded.